

Submission of amplicon libraries for Illumina sequencing

The CGR cannot guarantee good performance from externally prepared libraries.

Sample submission requirements

- Amplification conditions should be optimised to ensure synthesis of the expected product(s). In addition, we strongly recommend size selection of the samples before purification.
- Amplicon samples should be free from contaminants to prevent inhibition of enzymatic steps. Purify samples using columns or Agencourt AMPure XP beads and elute in nuclease-free water.
- After purification, each sample should be run on an agarose gel or automated electrophoresis instrument such as Bioanalyzer, TapeStation, Fragment Analyzer or equivalent, to evaluate quality. Please attach an image showing the results to the LIMS project submission form, including information about the ladder or markers used.
- Sample concentration should be determined using a dye-based method such as the PicoGreen or Qubit assays.
- Each library or library pool should be supplied in a tube labelled exactly the same as that given on the LIMS project submission form. If more than one tube is provided, please label them in numerical order for ease of sample identification. Please underline any numbers that could be misread upside-down (e.g. 6/9, 16/91).
- Please supply **≥100 ng DNA in a maximal volume of 30 µl nuclease-free water** per library. This will allow us to size select the libraries, if needed.

Experimental design

Please see the [standard Illumina sequencing primers](#) for an overview of the sequencing primers used for the Illumina platforms. Additional information on primers and adapter sequences can be found on the [Illumina website](#). If you wish to use custom sequencing primers, please contact us at CGR.Enquiries@liverpool.ac.uk to confirm their compatibility with our instruments and workflows.

When submitting your project in our LIMS, we request that you upload a document that outlines the entire sequence of the forward and reverse primers (adapter, index/barcode and template specific primer). Please highlight the barcodes/indexes on the document or provide them as a separate column for each sample.

If you are unable to meet the stated requirements for your library type, please contact us at CGR_Lab@liverpool.ac.uk and we will be happy to offer further advice.